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Thesis by *Major R. G. Archibald*

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K A L A A Z A R, WITH SPECIAL REFERENCE

TO ITS OCCURRENCE IN THE SUDAN

BY

MAJOR R. G. ARCHIBALD, D.S.O., R.A.M.C..

1919



I n t r o d u c t i o n .

The group of diseases caused by the parasites of the ~~genus~~ *Leishmania* has occupied the attention of numerous investigators in the ~~tropics~~ and elsewhere, and as the result of valuable researches, more especially during the last decade, considerable advance in knowledge regarding this group has been made.

The literature dealing with these researches has grown so extensively, that it has been no easy task to keep in touch with it, more especially, as a great deal of the work in recent years has come from the pens of investigators in other countries, and is published in many journals and in many tongues.

The group of diseases referred to comprises visceral Leishmaniasis, cutaneous Leishmaniasis, Espundia or naso-pharyngeal Leishmaniasis, and canine Leishmaniasis.

This paper deals more especially with the visceral form of leishmaniasis commonly known as Kala Azar, of which the following types exist a. Indian, b. Mediterranean or Infantile kala azar, c. Sudan type. The disease kala azar is also known in certain parts of India as kala dukh ; other synonyms for it are Tropical splenomegaly, black sickness, Sirkari disease, Sahib's disease, Burdwan fever, and Dum-Dum fever.

Historical account of the disease.

It first attracted attention in the year 1869 when the district of the Garo hills was occupied by the British. In 1875 it commenced to spread, became epidemic and caused a high death rate.

In 1882 the first account of the disease was

published by Clarke from notes of 120 cases compiled by McNaught
the civil medical officer of the district.

1

In 1889 Giles investigated it in Assam and concluded
that the disease was Ankylostomiasis.

In 1894 Stephens in his annual report stated that the
disease was allied to malaria.

2

Three years later Rogers reported it was malarial and

3

this was further supported by Ross in 1899.

4

In May 1900 Leishman found the causal parasite of the
disease in films taken from the spleen of a soldier who had died
in Netley from a fever contracted at Dum-Dum but did not publish
the account of his discovery till 1903.

5

In July 1903 Donovan
confirmed Leishman's observation obtaining the same parasite which
is now known as the Leishman-Donovan parasite.

6

In 1904 Christophers published a valuable report on the
parasites of the disease and in the same year Rogers observed the
development of the parasites into flagellate forms when inoculated
into special media and incubated at a low temperature.

7

8

In 1907 Patton showed that the parasites could be found
in numbers in the ~~leucocytes~~ ^{leucocytes} of the peripheral blood and further
that these parasites became flagellates in the alimentary tracts of
bed bugs.

9

In 1904 Neave in the Sudan discovered the existence of
the parasite in a child from the Bahr el Gazal province and in the
same year Philipps in Cairo discovered it in two cases from the
Yemen district in Arabia.

10

11

Cathoire in the same year obtained Leishmania parasites
in a child in Goulette in Tunisia, and in the following year

12

Pianese found the parasites in the liver and spleen of children
dying from splenic anaemia.

13

In 1907 Nicolle and Cassuto observed the parasites in the spleen of a child in Tunisia and named the disease Infantile kala azar. After this it was noted in Crete, in Sicily by Gabbi,

14

in Malta by Critien¹⁵, in Lisbon by Alveres¹⁵, while Gabbi proved that the disease known as "Ponos" in Spezzia was in reality Kala Azar.

Christomanos and others found the disease in the Grecian islands, while Batinos found it in Corfu. Kefalinos noted it in Pagos. About the same time it was found to be spread widely in Southern Italy.

18

Mavzinowsky observed cases in Moscow.

19

Ibrahim found

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cases in Tripoli, Lemaire noted the disease in Algiers and Sluka

21

and Zarfl reported cases in Turkestan.

Endemic centres of the disease have been noted in Peking, and the upper part of the Yangtse valley, and cases have been found by Elders²² and others in the Dutch East Indies occurring in natives and temporary residents.

Other infected areas may be mentioned viz. Syria, Ceylon, Burmah, Indo China. As might be anticipated diffusion of knowledge of the appearance of the parasite and of the methods necessary for its demonstration, led to the discovery of the disease in parts of the world remote from the East, showing that kala azar can no longer be considered as an entity of interest solely to those working in the tropics. Its distribution outside of India, where it was first seriously studied, brings one face to face with the problems of the identity, or non identity, of the Indian disease with other forms of kala azar, and much research has been carried out to elucidate this question.

Distribution of the disease in the Sudan.

In the Sudan the disease has a limited endemicity, occurring in remote districts difficult of access at certain times of the year, consequently

the facilities for scientific investigation have been somewhat arduous. Since Neave's discovery of the disease in the Sudan further cases have been recorded by Cummins²³, Bousfield²⁴ Thomson, Marshall²⁵ and the writer²⁶. Their investigations have shown that kala azar in the Sudan is comparatively uncommon, and usually occurs in the Sennar, Kagsala and Blue Nile districts.

A point of special interest is its relative frequency in villages closely situated to the Abyssinian frontier; where infected nomad Abyssinians have been found, and the question therefore arises whether the disease has been introduced to the Sudan from Abyssinia. On the other hand two cases have occurred in natives of the Western Sudan, where the disease was known among the Baggara and Darfur tribes. In the troublesome times of the Mahdi many of these Baggara Arabs raided the Eastern Sudan, and eventually settled in villages in the Blue Nile district, so it is *conceivable* ~~possible~~ that they brought the disease to the areas which are now endemic centres. The disease does not exist in ~~the~~ vast tracts of desert country, but appears to be limited to areas where there is a fairly extensive rainfall, and in villages dependent on rivers and khors for their water supply. Water seems to be connected in some way with the question of transmission of the disease in the Sudan.

In India kala azar has occurred in epidemic form more especially in Assam, where in 1900 there were no less than 6000 odd deaths. In the Sudan, however, it has never assumed an epidemic form even in the most heavily infected villages.

Evidence has been collected in India, which shows that the disease spreads slowly along channels of human intercommunication, and results from the introduction to a district of an infected individual.

Age incidence.

In India the disease may attack any age but shows a preference for young adults, whereas in the Mediterranean countries it is essentially a disease of infants, but in certain areas, it is known to occur in adults.

In the Sudan the disease affects people of all ages, but is commoner in late childhood, and early adult life.

Sex.

The disease is more common among the male population. ²⁷ Nicolle's statistics in Tunis show that boys are more commonly affected with the disease than girls.

In India the disease is more commonly seen in males, and numerous instances have occurred of the husband and wife becoming ²⁸ infected. Rogers has noted the extraordinary tendency of the disease to attack a number of persons in the same family. In the Sudan females rarely suffer from the disease, and no records exist of more than a single individual occupying the same dwelling contracting the disease.

Race.

No race or creed appears to be exempt from the disease, Europeans however rarely contract kala azar, but the enormous disproportion between them and the natives should be borne in mind.

A few cases have occurred among Europeans who have resided in endemic centres in the Sudan.

Seasonal incidence.

²⁹ In India the majority of the cases apparently become infected in the cold weather between the months of November and April. Gabbi stated that in Italy the greatest number of cases occur in March and April.

From evidence carefully collected by the writer the

majority of the Sudan cases become infected after the rainy season, between the months of August and December, a period which corresponds to the time when the natives are cultivating ; it has also been noted that a low rainfall is usually followed by a low incidence of the disease.

Etology.

The causal parasite is a herpetomon ^{as} L. donovani Ross 1903, and is found especially in the ~~endothelial~~ ^{endothelial} cells of blood vessels and lymphatics, spleen, liver, and bonemarrow, and in other organs. It is also present in the mesenteric and lymphatic glands, and in the ulcers occasionally found in the intestinal mucosa.

In the Indian cases the parasites have been frequently noted in the peripheral blood, more especially towards the end of the illness, and when intestinal symptoms are predominant. In the writer's experience however it is the exception to find the parasites in the peripheral blood even when Donovan's method of taking blood films has been employed.

Morphology of the Leishman-Donovan parasite.

A number of careful studies of the morphology of the parasite as encountered in the body tissues have been published. Such descriptions correspond closely with one another and confirm earlier impressions as to the great uniformity of structure observed in specimens taken from cases in different districts and countries. The parasite, as commonly met, with, occurs in the form of round or oval cells with a macro and a micro nucleus. Forms indicating the mode of growth and multiplication have been closely studied. Division appears to be commenced by amitotic splitting of the nucleus, which subsequently extends to the cytoplasm.

The large number of parasites found in the cells has given rise to discussion as to how large a part is played by

phagocytosis. It appears probable that the first parasites are taken into the cell in this manner, but that intracellular multiplication will account for the larger number. Differences in size and shape of the parasites have been mentioned in relation to the organ from which they were derived.

Pianese ³⁰ for instance considered they were smallest in the liver, and largest in the bonemarrow, while those in the spleen were of intermediate size; from this he suggested that the marrow was probably infected first, and the liver last, but other observers do not confirm this difference in size. The bone marrow however appears to form the most favourable medium for the parasite to thrive and multiply.

As seen in the Sudan the parasite is remarkably constant in form and shape, and when compared with the parasites of Indian or Mediterranean kala azar shows no distinguishing features which might serve towards a differentiation ^{ion} of species. (Plate I.).

In the acute types of the disease the writer has noted that the parasites are more rounded than those seen in the more chronic types of the disease and fission forms are commonly met with.

Bousfield ³¹ comments on the occurrence of parasites in the liver or spleen of cases in the Sudan in which the two ~~chromatin~~ ^{chromatin} masses appeared of nearly equal size, and suggests the possibility of these being sexual or conjugative forms; but this appears unlikely and parasites are not infrequently seen, where such an enlargement of the centrosome, is obviously an early stage of simple fission of the parasite.

Cultures and cultural forms of the parasite.

The first successful cultural experiments of Rogers (32) were soon

repeated and confirmed by Leishman and Statham and since then by many others and successful development of the parasite into flagellate form is usually to be obtained by keeping citrated splenic blood under aerobic conditions at a temperature of 22°C-25°C.

Success however is by no means invariable, even when the parasites are fairly abundant and when the material is derived directly from a splenic or hepatic ^{puncture,} ~~nodule~~ and not obtained post mortem.

Rogers' recommendation that the cultures should be acidified by the addition of a small quantity of citric acid does not appear to be ^{so} ~~as~~ advantageous as that observer would make out.

³⁴ In material obtained from cases of Sudan kala azar the writer obtained successful cultivation of the parasite at 22°C in Novy-McNeale-Nicolle medium containing defibrinated rabbit's blood. Defibrinated sheep's blood was employed as a substitute but without success; it appeared to exercise an inhibitory influence on the growth of the flagellates of L. Donovanii, although proving a favourable medium for the pansilea of L. tropica. Cultures incubated at 22°C were also obtained on ox blood serum and Buchanan's glucose neutral red media. Novy McNeale Nicolle (NNN) media appeared however to be the most suitable. Infected material placed in this medium showed at the end of 6 hours slight increase in size of the parasites. At the end of 12 hours there was a further increase, with enlargement of the macro and micro nucleus. At the end of 24 hours the parasite was more elongated with the macro nucleus occupying a central position and the micro nucleus appearing as a transverse rod at the anterior extremity of the body with a small flagellum emerging from it. During the next 24 hours the parasite and its flagellum increased

in size and showed marked ^{motility} ~~motility~~. Further changes consisted of the development into the adult flagellate multiplication by division and rosette formation. The body of the adult flagellate measured 14 to 21 microns in length and 1 to 2 microns in breadth while the flagellum averaged 16 to 24 microns in length. In 6 to 10 days the parasites had reverted to their original type having lost their flagella and become thick walled oval cells representing the so called cystic stage. (Plate 2.).

It was found that development into flagellates could occur under anaerobic conditions ; aerobic cultures however produced a richer growth of flagellates.

Bacterial contamination of the cultures invariably ended in the arrest of development and death of the flagellates.

The optimum temperature appeared to be 22°C .

The parasite of Infantile kala azar (*L. infantum*) grows readily in NNN media whereas the parasite of the Indian strain grows with difficulty and this cultural difference was held to be one of the more important points in support of the view of the duality of species of Indian and Mediterranean kala azar.

35

Row recommends as a culture medium defibrinated rabbits' blood to which is added water and sodium chloride@

Cytological characters of the flagellates.

To study these young cultures were employed so as to obtain dividing and not degenerated forms.

The preparations were ~~not~~ fixed and ~~stained~~ either by Iron haemotoxylin, and then differentiated with a solution of Iron alum, or were stained by Giemsa and differentiated with orange tannin.

By these methods of staining the main characters of the

macro and micro nucleus could be noted. The macro nucleus was vesicular in type, and limited by a well defined nuclear membrane containing on its inner surface chromatin dots, which were well marked in dividing forms of flagellates. In the nuclear sap zone there was an intensely stained karyosome, but no centriole.

Between the ~~macronucleus~~ ^{macronucleus} and the anterior extremity of the body the karyosome of the micro or kinetonucleus could be seen, and close to it a basal granule, from which the rhizoplast took origin. In some of the flagellates the basal granule was represented by a thickening of the rhizoplast.

In the dividing forms the process of fission usually commenced in the basal granule, and was followed by division of the rhizoplast and flagellum.

At the same time the karyosome of the kinetonucleus elongates and then divides, and this is followed by division of the nuclear ~~chromatin~~ ^{chromatin} of the macronucleus. Some forms are observed in which division of the macronucleus precedes that of the kinetonucleus.

Symptomatology.

Although nothing is known of the exact incubation period of Kala Azar, from the evidence collected, it would appear that it varies from ~~100~~ 10 days to three weeks or several months. In the Sudan cases it has been difficult, for various reasons, to obtain accurate data but such evidence as has been produced, permits one to place the incubation period about 60 days, a period which corresponds more or less to the incubation period in experimentally infected monkeys.

The onset of the disease is usually insidious commencing with attacks of fever of a remittent type frequently showing in a four hourly temperature chart two remissions per diem. As pointed out by Rogers (36) this double remission in the 24 hours

KALA-AZAR

KALA-AZAR

10



Temperature chart illustrating the Rembrandt type of storm in Indian Wells Pass,
or the inefficiency of Quinine in the treatment.

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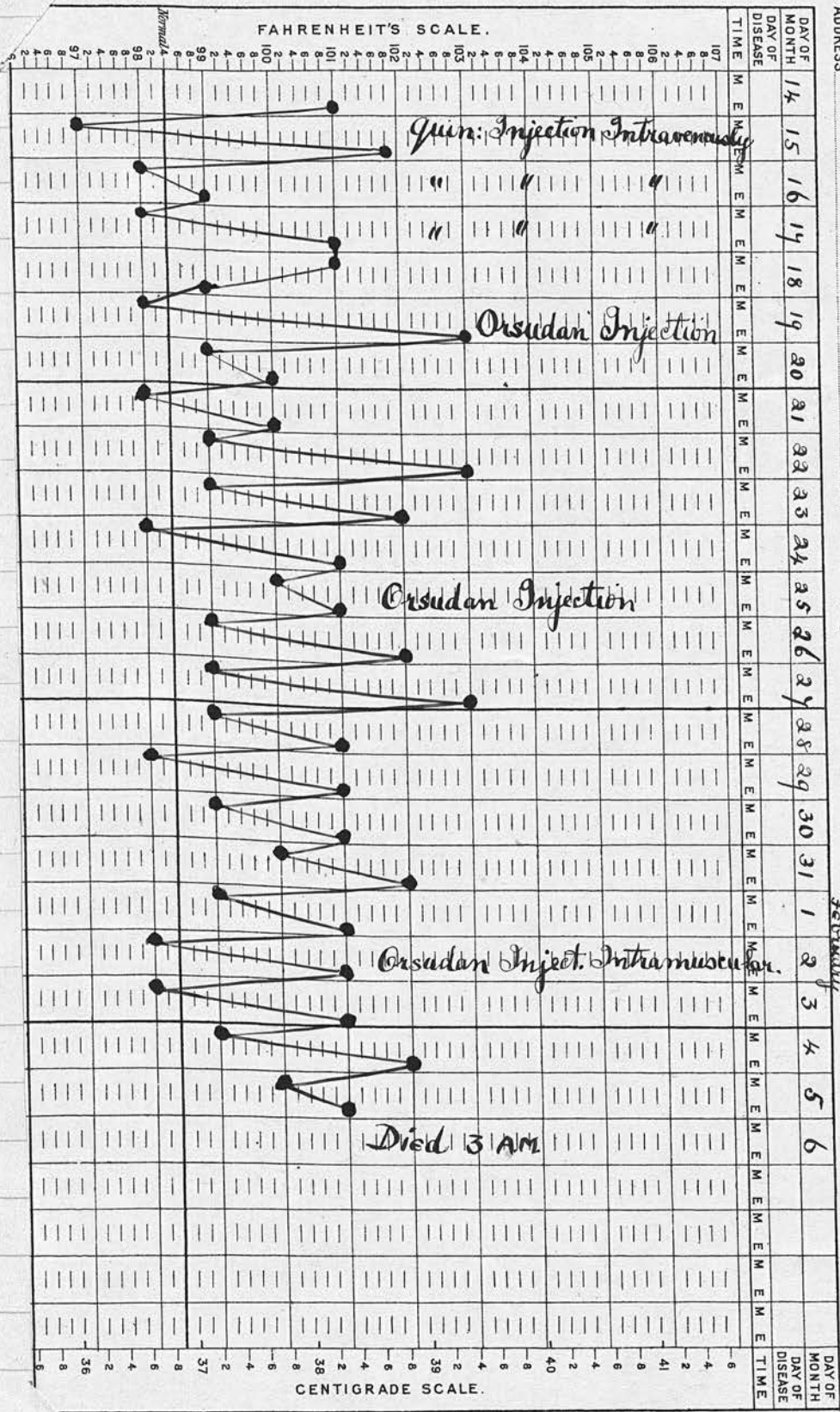
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February

DISEASE

KALA-AZAR

JAN: 1918.



Sudan Kala Azar.

is diagnostic of the disease.

Usually towards the end of the 3rd. or 4th. week the fever declined but at this period the spleen and liver show some enlargement.

The decline of the fever is followed by an interval of apyrexia, which may last some weeks, and this in turn is succeeded by a further period of fever, with shorter intervals of apyrexia till eventually the fever assumes a continued type, and is associated with cachexia and progressive emaciation. In the more chronic forms of the disease with great enlargement of the spleen and liver the fever is of a low intermittent type. Headache and rigors may accompany the febrile periods.

The pulse rate is invariably accelerated, and in the later stages of the disease is feeble and of a low tension.

Spleen.

Every case has shown an increase in size of this organ, varying from a slight enlargement below the costal margin, to a point, several inches below the umbilicus.

On palpation, these enlarged spleens are found to possess a characteristic consistence, which can be detected with experience. They are softer than spleens met with in chronic malaria or syphilis.

In India Rogers has noted that the enlarged spleen varies in size, more especially towards the fatal termination of the disease; this however has not been noted among the Sudan cases.

Liver.

This organ is invariably enlarged, and in the more chronic cases is often associated with ascites.

Blood changes.

These are severe, and consist in the main, of a progressive anaemia.

There is a diminution in the number of red blood corpuscles, usually not lower than 3 million per cubic millimetre. Microcytes, megalocytes and poikilocytes are frequently present; in the advanced stages of the disease, nucleated red cells may be found.

Associated with the destruction of the red blood cells there is a relative leucopenia, which is so constant as to serve as a diagnostic feature of the disease. This has been noted by Donovan³⁷ and Brahmachari³⁸ in Indian kala azar but is less constant in infantile kala azar. In the Sudan cases the leucocytes are frequently reduced to 2000 odd per c.m.

In a typical uncomplicated case the polymorphonuclear leucocytes are diminished in numbers, while the large mononuclear leucocytes and large and small lymphocytes are increased. A characteristic feature too noted by the writer, is the marked diminution and often complete absence of eosinophiles.

A differential leucocyte count in a typical case is usually as follows

Polymorphonuclear leucocytes	30 per cent.
Large lymphocytes	16.6 , , ,
Small lymphocytes	37.0 , , ,
Large mononuclears	14.2 , , ,
Transitionals	2.2 , , ,
Eosinophiles	nil.

Haemoglobin.

This is always reduced, the reduction corresponding to the oligocythaemia present.

Coagulability of the blood.

Even in the early stages of the disease the blood shows a diminished coagulability, and this probably accounts for the epistaxis which has been noted so frequently.

Alkalinity of the blood.

Researches carried out some years ago by the writer showed, that

the alkalinity of the blood was diminished in this disease.

This observation has since been confirmed in India by Rogers.

Intestinal Symptoms.

In the early stages of the disease there is constipation associated with alternating attacks of diarrhoea. In the advanced stages diarrhoea is constantly present in the Sudan cases. The stools are usually acholous, and often contain blood and mucus, and microscopically may show the presence of pathogenic entamoebae.

Cutaneous affections.

Pigmentation of a brownish yellow colour, more especially of the skin of the face, is occasionally observed in the Sudan cases. It is often difficult to detect ^{it} in the dark skin of natives.

Vesicular and bullous eruptions have been recorded in other countries, but have not been noted in the Sudan.

Oedema.

In advanced stages of the disease oedema of the ankles and eyelids is often present.

Urinary system.

Albuminuria is occasionally present.

Complications.

In other countries pneumonia, skin ulcers, haemorrhages, cancrum oris, and dysenteric symptoms have been noted.

In the Sudan cancrum oris followed by septic pneumonia has been observed in a few cases ; as a rule the progressive anaemia and asthenia cause a fatal termination to the illness before complications set in.

Types of the disease in the Sudan.

Two main types exist (1) Acute occurring in children and young

adults (Plates 3 & 4.), and usually terminating fatally in three to six months.

Clinically such cases show great wasting, marked enlargement of the spleen and liver, associated frequently with ascites, abdominal distension. (Plate 5.).

The disease in adults runs the same course as in children and there appears to be no justification in differentiating it.

A number of cultural and animal experiments have been carried out by the writer with strains of parasites obtained from children and adults, and the results have been very similar.

(2). Chronic type chiefly seen in adults associated with spleens - megaly and a low intermittent type of fever. The wasting and abdominal distension is less marked than in the acute type. Such cases usually live for one or two years till some intercurrent malady carries them off. They represent ambulatory types of the disease, and being carriers of the parasite prove a source of danger to any community.

Cultural and animal experiments with a strain of parasites obtained from one case, were similar in results, to those obtained in the acute cases.

The inference is, that in the chronic type of the disease the infection has resulted in a greater immunity on the part of the patient.

Intercurrent maladies.

Although kala azar is endemic in areas where malaria is prevalent, it has been the exception in the Sudan to find the two diseases existing in the same individual.

In only two instances the writer found malarial pigment, but no plasmodia in splenic films from cases of kala azar.

In one case ankylostomiasis was noted, while in another

case of kala azar microfilaria bancrofti. was present.

The faeces of kala azar cases have been repeatedly searched for ova of intestinal parasites with negative results except in the above mentioned case of ankylostomiasis.

Eutamoeba histolytica, *Trichomonas*, *Enteromonas* and *Chilomastix* flagellates have occasionally been found.

Morbid anatomy and pathology.

Emaciation, muscular atrophy and oedema have already been mentioned.

The intestines are usually pale in colour, and present a peculiar blanched or tissue paper appearance, more especially in advanced stages of the disease.

Ulceration of the large intestine is not commonly present in the Sudan cases. In one autopsy the ulceration (Plate 6.) of the large intestine was extensive, and macroscopically resembled the lesions present in bacillary dysentery. Microscopical examination showed degenerated *Leishmania* parasites present.

Intestinal ulceration has been noted in the Indian and Mediterranean types of the disease.

Mesenteric glands.

The mesenteric glands are usually congested, and ⁴⁰ *Manson*, *Low* and others have found parasites in the mesenteric glands. The writer however has failed to demonstrate them in the glands of Sudan cases.

Spleen.

Reference has already been made to the enlargement of this viscus being invariably present.

Macroscopically the organ is of a characteristic deep red colour, and of a consistence not as firm as seen in chronic malaria.

The capsule is usually thickened, and beneath its surface

infarcts have been noted in a few cases.

Microscopically, sections show a general congestion of the organ with numerous macrophages and large mononuclears containing parasites. In the more advanced and chronic cases evidence of a general fibrosis is usually present.

Liver.

The enlarged organ is usually of a firm consistence, has a smooth surface, and a nutmeg appearance.

Sections show dilation of the intralobular^u capillaries, with macrophages derived from their endothelial wall, containing phagocytosed parasites.

In some cases an intralobular cirrhosis causing atrophy of the liver cells is present, a condition which has also been noted by Rogers in cases of Indian kala azar.

Bonemarrow.

The bone marrow is usually soft and diffluent, and of a reddish colour, and contains numerous parasites free and phagocytosed. It appears to form the most suitable medium in the body for the ^{growth} development of the Leishmania parasite.

Transmission of the disease.

The question of transmission has been productive of a vast amount of research.

Investigators in different countries have evolved various hypotheses often supported by strong experimental evidence, but many of these have been open to criticism, or have broken down on epidemiological grounds, and the fact remains that the problem still remains unsolved. Its solution in the Sudan has been rendered more difficult than in other countries by reason of the absence of epidemics of the disease, and the consequent lack of sufficient clinical material for a prolonged series

of investigations. Further, owing to the remoteness of the endemic centres, it has not been possible to establish there facilities for laboratory work.

The investigations carried out by the writer have been conducted chiefly in Khartum - a non endemic area - and therefore suitable for animal experiments.

Previous to carrying out these researches the writer had made a long tour of inspection along the Abyssinian frontier, and obtained as much epidemiological data as possible from villages where the disease was known to exist, and in the course of this inspection had inoculated susceptible animals, and had carried out a large number of dissections of haematophagous insects, and moreover examined the blood and organs of domestic and wild animals living in these areas.

Probable modes of infection.

- A. By means of the bites of haematophagous insects and diptera.
- B. By oral ingestion.
- C. By soil infection,
- D. By helminths.

A. By means of the bites of haematophagous insects and diptera.

Much of the research carried out to prove the rôle played by blood sucking insects and flies has been open to criticism owing to the fact that investigators had failed to realize that many blood sucking insects were natural hosts for parasites of the herpetomonad group, consequently, a good deal of evidence incriminating these insects has now broken down, and will lead to researches being carried out on fresh lines, still it will be useful to review and consider the possible rôle of suspected haematophagous insects, and diptera, concerned with the transmission of this disease.

1. Bed bugs.

42

Patton was the first to discover the development of the Leishman-Donovan parasite in the bed bug *C. rotundatus*, and the view that this insect was the transmitting agent of Indian kala azar was for a time generally accepted.

The theory of bug transmission received support on epidemiological grounds, ^{also} and from the results of the special prophylactic ^{measures} ~~measures~~ carried out by Dodds Price ⁴³ but Donovan and others obtained no evidence to support it.

Donovan examined the gut contents of a large number of bugs obtained from areas where the disease was endemic without encountering any *Leishmania* parasites.

44

More recently Cornwall and his co-workers have confirmed Patton's observations, in as much as they recovered flagellates of *L. donovani* from the stomach of infected bugs. They failed however, to recover viable forms of the parasites from the faeces of the insects.

45

In further experiments Cornwall showed that the bed bug could not regurgitate its stomach contents, and therefore was incapable of transmitting kala azar.

For the bed bug theory to remain tenable, it would appear necessary to postulate some further development of the parasite, possibly a flagellate or granule form, which infects the ovary of the bed bug and so brings about a hereditary transmission; this would certainly explain the very localized zones of intense infectivity which form such a feature of the epidemiology of the disease.

In the Sudan the bed bug *C. lectularius* is ubiquitous while *C. rotundatus* is only occasionally found.

Both species have been used in feeding experiments but *Leishmania* parasites were never found in their stomach contents. It should be remembered however, that in the Sudan, it is the exception to find *Leishmania* parasites in the peripheral blood so that it is possible that the bugs employed in these feeding experiments remained uninfected.

On another occasion *C. lectularius* bugs obtained from native beds occupied by kala azar cases were dissected, and their stomach contents injected into a susceptible animal with negative results.

2. *Conorrhinus* bug.

46

Donovan suggested that the plant feeding and occasional blood sucking bug *Conorrhinus rubrofasciatus* was a possible transmitting agent. This theory rests on somewhat slender evidence.

47

Cornwall in some recent experiments showed that the parasites in flagellate form did not flourish within the stomach of this bug.

48

Wenyon in a critical paper states, that the development of *L. donovani* in bugs does not prove that these insects are transmitting agents.

49

Mackie has examined a very large number of bugs from infected centres in India, and failed to obtain evidence incriminating these insects.

Fleas.

50

Nicolle showed that *L. infantum* is a natural disease of dogs and

51

Pianese and others proved the transmission of this disease from dog to dog and from dog to man by the agency of the dog flea

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P. serraticeps and *P. irritans* Basile and Sangiorgi "

discovered what appeared to be *Leishmania* parasites in fleas taken

~~Many~~ infected dogs, and carried out successful transmission experiments in dogs.

These experiments led to the view that the Mediterranean type of kala azar (*L. infantum*) was transmitted from dogs to man by the agency of fleas, and evidence steadily accumulated showing that dogs ^{were} ~~are~~ natural hosts of *Leishmania*, and frequently suffered from canine leishmaniasis.

Exception however has been taken to the results of a large number of experiments that implicated the flea, on the grounds that (a) fleas naturally harbour flagellates of the herpetomonas group (b.) canine leishmaniasis is a different entity from human leishmaniasis.

54

Moreover Spagnolio has recently collected evidence which throws some doubt as to whether dogs are in any way connected with the disease.

55

Gabbi's feeding experiments with the human and dog flea afforded negative evidence, and this observer contends the theory of flea transmission.

A number of observers have failed to infect fleas with *L. donovani*, and further, in India Donovan and Patton have failed to find dogs naturally infected with *Leishmania*.

56

57

In the Sudan the writer has failed to obtain *Leishmania* parasites in *P. canis* taken from dogs inhabiting endemic areas, and inoculation experiments with stomach contents of dog fleas have proved negative. Moreover, further dogs are not very susceptible to experimental inoculation with the Sudan strain, and none of these animals have been found to be naturally infected.

Lice.

58

Researches carried out with the body and head louse by Patton and Mackie have proved negative.

Franchini too has stated that *L. donovani* cannot live in the digestive tract of lice.

Feeding, and inoculation, experiments, likewise dissections, have proved negative in the Sudan.

Ticks.

59

In India these have not been incriminated. Patton's feeding experiments with *O. savignyi* proved a failure. The writer has had similar results with *O. moubata*, *O. savignyi* and ^{*argas*} ~~*argas*~~ *brumpti*; the last mentioned tick has been found in endemic centres in the Sudan.

Dissection and inoculation experiments with the common dog tick *R. sanguineus* have proved negative.

Mosquitoes.

60

Franchini's researches show that *L. donovani* is able to live and develop in the digestive tract of *A. maculipennis*, and this observer concludes that mosquitoes play an important rôle in the transmission of kala azar.

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Patton's and Mackie's experiments with *Culex*, *Anopheles*, and *Stegomyia* proved negative,

63

Wenyon favours *Stegomyia fasciata* as a possible transmitting agent, as he found that *L. tropica* could live and develop in this mosquito.

In the Sudan some researches have been carried out on a small scale with *F. costalis* but these proved negative.

Franchini's experiments implicating the mosquito have not been confirmed. Exception has been taken to them on the

grounds that the mosquitoes employed by this observer may have ,
harboured flagellates of the herpetomonas species.

Phlebotomus flies.

Few researches have been carried out with these flies.

64

Mackie examined several in India and found 10 per
cent infected with a herpetomonad flagellate.

In the Sudan, *P. minutus* and *P. pappatasi* are commonly
found. Dissection of a large number of these proved
negative.

House or domestic flies.

There is no evidence to show that the common house fly can transmit
the disease.

In the Sudan there is strong circumstantial evidence
implicating *S. calictrians* as a transmitter of *L. tropica* but this
fly has not been noted in the endemic areas of kala azar.

B. Transmission by oral ingestion,

65

The writer a few years ago considered the possibility of
infection occurring via the digestive tract, and successfully infected
two healthy monkeys (*L. callitrachus*) by means of carefully conducted
feeding experiments. One monkey became infected in 36 days
while the other showed infection in 132 days. (Plate 12.)

66

Basile a year later obtained positive results in
similar feeding experiments. The possibility of such a
method of infection should not be lost sight of and the writer is
of the opinion that it may occur by the ingestion of some water
insect eg cyclops serving as a natural host for the *Leishmania*
parasite.

The question whether the parasite exists as a
commensal in the cytoplasm of intestinal flagellates has yet to

be proved, so far there is little or no evidence to support such a view.

With regard to the method of infection by the intestinal tract it is of interest to note that Farnham and Porter⁶⁷ were successful in parasitizing certain vertebrates by feeding them with fleas, lice and other blood sucking insects infected with herpetomonads.

3. Transmission by infected helminths.

Knowles⁶⁸ in a recent paper discusses this possible mode of infection. Helminths were noted in 42 cases of kala azar but no evidence was produced to show that taenia, ascaris, trichina or ankylostomes were ever infected with Leishmania parasites.

In the Sudan they cannot be suspected for they have rarely been found in kala azar cases.

4. Infection from contamination of soil and water and food.

The theory of localized soil or site infection has always attracted some workers. In India Dodds Price showed that the removal of an infected family 300 yards from their former residence invariably arrested the spread of the disease. Further it was noted that in Assam infants in arms were never attacked with the disease. Was this associated in any way with their inability to visit the family latrine patch? Such a view of possible soil infection is interesting but can hardly be reconciled with our knowledge of the bionomics of the Leishman-Donovan parasite - a parasite endowed with but feeble properties of resistance^{and one} barely fit to withstand such powerful and inimical agencies as heat, strong sunlight, and the presence of organisms unfavourable to its existence.

For the same reasons the theories of water and food

infection will scarcely hold good. Moreover Dodds Price⁶⁹ and Rogers have collected evidence, showing that the theory of either water or food infection fails to explain the spread of Indian kala azar.

Experimental kala azar in animals.

Stress has been laid on animal experiments serving as differentiating tests for the various types of kala azar ; how far such tests should be considered in such a light remains open to question, for the conditions favouring experimental inoculation may show considerable variation even when one is dealing with the same strain of virus.

Nicolle's inoculation experiments in dogs and monkeys with the infantile strain of kala azar have been positive in the majority of instances, but Italian and French investigators have not been able to secure infection in anything like the same proportion of animals.

The earlier attempts to inoculate animals with the Indian strain proved unsuccessful, owing to the fact, that too⁷⁰ small doses of the virus were employed. Donovan⁷¹ was the first to inoculate successfully a dog with the Indian strain, and it is interesting to note that in this experiment parasites were only present in the bone marrow.

Donovan's results were confirmed by Wenyon⁷², Patton⁷³, and others.

Row⁷³ obtained interesting results with the small variety of *Macacus sinicus*, and was able to produce a general infection with the virus of Indian kala azar ; in another animal of the same species inoculation was followed by a local lesion without a general infection ; the local lesion resembled that of

E. tropica.

M-cynomolgus monkey has also been successfully infected with the Indian virus.

Row found that the strongest infections with animals were produced by using old cultures, intraperitoneal inoculation causing a general infection, and subcutaneous inoculation ~~sometimes~~ producing sometimes a local and at other times a general infection.

The following animals have been successfully inoculated with the Indian virus, dog, jackal, macacus monkey, flying fox, white mice and white rats, whereas the guinea pig, rabbit, kitten, goat, young pig, gekkos and lizards have proved refractory to infection.

The virus of Mediterranean kala azar has been successfully transmitted to the dog, jackal, different species of monkeys, white mice and rats, guinea pigs and rabbits.

It should be noted however that guinea pigs and rabbits are infected with difficulty.

As regards animal experiments there appears to be little difference between the Indian and the Mediterranean virus.

74

Laveran's experiments showed that a macacus monkey which was immunized against the Mediterranean virus was also immune for the Indian virus. Laveran considers that the result of this experiment furnishes strong proof in favour of the Indian and the Mediterranean virus being the same.

75

In the Soudan the writer has shown that the grey monkey (*E. callitrichus*) pup, jerboa and gerbil can be successfully infected. It should be noted however, that dogs and pups are not very susceptible, intravenous inoculation sometimes failing to

infect these animals.

In the gerbil and jerboa the disease apparently runs a chronic course, in no way impairing the health of these animals.

Guinea pigs, rabbits, cats, kittens, pigeons, white rats and white mice appeared to be refractory to the Sudan strain of kala azar.

In a recent experiment the writer successfully vaccinated a grey monkey with infected splenic material from another monkey.

The skin over the left eyebrow was vaccinated, and 63 days later a local lesion containing Leishmania parasites was present. (Plate 7 and 7A). The animal at the end of three months showed anaemia, and spleno-megaly, and was killed on the 133rd. day. The local lesion showed parasites at the time of death, and the spleen, liver, and bone marrow, were also found heavily infected.

In another experiment a general, as well as a local, infection was produced, in a grey monkey with a subcutaneous inoculation of infected, spleen, liver and bone marrow. (Plate 8)

The results of these two experiments correspond closely to those obtained by Row in India with another species of monkey.

The disease in experimentally infected animals.

In susceptible animals such as the monkey the virus causes a progressive anaemia and wasting, with enlargement of the spleen, and slight enlargement of the liver. (Plate 9.).

In the Soudan, the writer has failed to find the parasites in the peripheral blood of infected animals. The spleen, and liver, more especially the former, show numerous

parasites, but they are found in larger numbers in the bone marrow, which is usually red and diffuent.

As already mentioned the incubation period in monkeys is usually about 60 days.

Natural hosts of the parasite of Sudan Kala Azar.

1. Animals and birds.

A large number of wild and domestic animals and birds have been examined with negative results.

Particular attention was paid to the dog, but in spite of numerous post mortem examinations of healthy and diseased dogs, no evidence of Leishmaniasis was found in these animals. In the endemic centres of the disease the dogs are particularly prone to infection with *Siroplasma canis*.

2. Insects.

As already mentioned under the subject of the transmission of the disease, dissections of numerous haemotophogous insects have yielded negative results as regards the presence of Leishmania parasites.

76

Balfour has recorded the presence of crithidia flagellates in the flea of the gerbil, *Xenopoylla Cleopatrae*, and the writer has found herpetomonad flagellates (Plate 10.) in the plant bug *Lygaeus militaris*. 78 Aders also noted the occurrence of a herpetomonas parasite in the melon bug *Aspongopus viduatus*.

Biological experiments with cultures obtained from cases of Sudan kala azar.

These were carried out with the object of obtaining any facts which might have a bearing on the transmission of the disease.

A resumé of these experiments may be given here.

The effect of sunlight.

Twelve hours exposure of a 7 day old culture of flagellates resulted in the flagellates reverting to ~~thick walled~~ oval forms or cysts.

The effect of temperature.

Temperatures from 31°C to 41°C resulted in the flagellates assuming cystic forms, which could develop again into active flagellates, when the culture was incubated at 22°C . A temperature of 50°C for half an hour resulted in the death of the flagellates.

The effect of hydrochloric acid 0.2 per cent..

This experiment was intended to represent the action of the gastric juice on cultural forms of Leishmania. A mixture containing equal parts of culture and 0.2 per cent hydrochloric acid was examined at intervals. At the end of 5 hours active flagellates with well marked translatory movements were present.

The effect of tap water and sterile distilled water.

Mixtures of tap water and culture kept at room temperature 80°F . showed at the end of 5 hours active flagellates, and a number of cystic forms.

Under the same temperature conditions sterile water had the little effect on cultures. At the end of 36 hours active flagellates and cysts were noted.

The effect of river water.

River water with a bacterial count of 220 micro-organisms per cc, converted all the flagellates into cystic forms, when such a mixture was kept at 22°C .

The effect of human serum.

Equal parts of human serum and a six days' old culture of flagellates when mixed, resulted in the flagellates immediately becoming motionless as if killed in situ.

This observation was also confirmed by Cornwall and Lafrenais, who attribute this inimical effect of the human serum to the action of its complement.

The inference drawn from these few experiments is that the cultural forms of *L. donovani* (Sudan strain) possess greater powers of resistance than commonly supposed. Under unfavourable conditions short of immediate death, the flagellates tend to revert to small oval forms with thick walls, which may be aptly likened to cysts.

Anomalous types of Kala Azar.

Such types undoubtedly exist and probably are more common than usually supposed.

Dodds Price has called attention to cases of splenomegaly in which parasites could not be found in splenic puncture films. In some of the cases however they were noted in the peripheral blood. Price suggests that these cases were in an early stage when infection was taking place and that they represented an anomalous type of the disease.

The writer⁸⁰ recorded some years ago an interesting case which represented an anomalous type of the disease, and which eventually recovered; clinically, the case resembled one of kala azar but repeated spleen and liver punctures failed to demonstrate the parasite. Peculiar coccal bodies were noted in the liver (Plate 11). An emulsion of spleen and liver material obtained from this case, when inoculated into a monkey, produced a kala azar infection.

These intracellular bodies have been found by the writer in another case of kala azar which eventually recovered, and were also found in the liver smears of a pup used in kala azar feeding experiments.

81

Smallman has also noted similar bodies in a case

82

of Mediterranean kala azar and Statham and Butler have observed them in a case of splenomegaly in Sierra Leone.

Diagnosis

This is based on the finding of the parasites in the blood or organs of the infected individual.

Short of laboratory aid a tentative diagnosis may be given from the temperature, which shows a double remission daily, and which is not influenced by the administration of quinine.

Diagnosis from the peripheral blood.

The existence of anaemia, with a relative leucopenia, an increase of large mononuclear leucocytes, and an absence or marked diminution of eosinophiles

is pathognomonic of the disease. The causal

parasites may be present in the peripheral blood, as has been noted by various Indian observers.

In the Sudan the writer has rarely found them although

83

Marshall in a few cases was more successful.

Cultivation of the parasite from the peripheral blood.

84

This was successfully carried out by Mayer and Werner and later

85

by Wenyon and others ; and is a useful aid to the diagnosis

86

Cornwall has pointed out the necessity of making a large number of cultures from the peripheral blood, in view of the paucity of parasites present.

Spleen and Liver puncture.

In cases of doubt, spleen puncture is the more preferable, as this organ is usually richer in parasites.

Owing to the risk of haemorrhage, the operation is attended with a certain degree of risk ; to minimize this, the patient should be given Calcium lactate in 15 grain doses on the day previous to the operation. In carrying out the operation the writer

employs a sterile and dry hypodermic syringe with a needle possessing a fine bore. After the operation, a binder is firmly applied to the abdomen.

Splenic puncture is contra indicated if severe bronchitis be present.

In fatal cases the parasites quickly degenerate in the organs, rendering diagnosis difficult, in such cases, recourse should be had to the bone marrow, where typical parasites can usually be found several hours after death.

As an alternative to spleen or liver puncture pustulation of the skin has been recommended by Cummins ⁸⁷ parasites being found in the leucocytes present in the exudate.

Differential diagnosis.

The following diseases may simulate kala azar.

1. Enteric fever. In its early stages kala azar has been mistaken for Enteric fever, but blood cultures, agglutination tests, ^{ina} and bacteriological examination of the faeces and urine will aid the diagnosis. 2. *Malta Fever*. *Haemoculture, serum tests & liver puncture* ^{will aid diagnosis}

3. Chronic malaria. Considerable difficulty may be experienced in distinguishing the two diseases. The periodicity of the fever, especially if it responds to treatment by quinine, the presence of malarial parasites, or pigment, and an eosinophilia will serve as distinguishing features, but often recourse to a splenic puncture is necessary, to prove the presence, or absence of kala azar.

88

4. Infantile splenomegaly with pyrexia as recorded by Nicolle and others, may cause difficulty, in diagnosis, necessitating a splenic puncture to prove the absence of Leishmania parasites.

5. Banti's ⁸⁹ disease characterized by splenomegaly, cirrhosis of

The liver and anaemia simulates kala azar. It has been
 90
 recorded in Egypt and the Sudan, where observers have had to
 carry out splenic puncture to aid the diagnosis.

6. Splenomedullary and lymphatic leucaemia and splenic anaemia
 may simulate kala azar. Examination of the peripheral blood
 is usually sufficient to differentiate these diseases.

7. Splenomegaly of syphilitic or tubercular origin may resemble
 the splenomegaly of kala azar. The signs and other symptoms
 of these diseases usually present will aid the diagnosis.

Mention may be made of a fatal case of *Toxoplasmosis*
 91
 simulating kala azar recorded by Castellani and the cases of
 92
Histoplasmosis observed by Darling at Ancon. Darling's
 cases showed irregular fever, splenomegaly, and a leucopenia caused
 by parasites of the *Blastomyces* group.

Prognosis.

The prognosis in kala azar is generally serious. Certain cases
 93
 however do recover, but they form a small percentage. Rogers
 has noted that complete recovery may occur in cases complicated
 with cancrum oris or pneumonia, and attributes this happy result to
 the great increase of leucocytes in the blood.

94
 Caronia and Cristina noted in two recovered cases the
 presence of specific amboceptors, and Caronia considers that
 recovery in certain cases results from the development of specific
 immune substances in the blood.

In the Sudan the disease is invariably fatal. A few
 cases of recovery, however, have been observed by the writer, who has
 noted in all such cases a steady increase of the eosinophile
 ratio in the blood.

Prophylaxis.

The lack of accurate knowledge regarding the method of propagation

of kala azar renders it difficult to set down rules and regulations for prophylaxis.

95

Rogers was the first to recommend segregation, a procedure which has been amply justified, by the results obtained in Assam by Dodds Price, who also recommends the disinfection of infected houses, and the removal of infected villages to fresh sites.

The exact rôle played by dogs is not clear ; the facts however that dogs can be infected experimentally, suggests that measures be directed against these animals.

Protection against the bites of blood sucking insects, and the prevention of the emigration of individuals from endemic areas appear measures worthy of adoption.

Treatment.

96

Gaspar Vianna in 1913 was the first to use intravenous injections of tartar emetic in the treatment of American leishmaniasis, and since then this drug has been universally employed in kala azar with brilliant therapeutic results.

97

In 1914 Castellani treated a case of kala azar with intravenous injections of tartar emetic, combined with iodide of potassium, and Fowler's solutions, and obtained good results.

The value of tartar emetic in the treatment of Mediterranean kala azar was proved by Dicristina and Caronia who reduced the mortality from this disease by 80 per cent.

98

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100

Rogers and Hume Mackie, and others have proved its value in the treatment of Indian kala azar, and Christopherson has reported favourably on its use in Sudan kala azar.

With all this evidence there can be no doubt that antimony, and its salts, are specific remedies for leishmania infections, more especially when given intravenously. By this method more certain results are obtained but care is necessary, that the

drug enters direct into the selected vein, and not into the surrounding tissues.

Two to ten cubic centimetres of a sterile one per cent solution of antimonium tartaratum in warm normal saline are injected intravenously, for five to ten days, and then every other day, and finally once a week. For children under one year $\frac{1}{2}$ - 1cc should be given daily for seven days, and for children one to five years of age 1 - 3 cc is recommended.

Before and during treatment the urine should be examined for evidence of albumin, and as Knowles¹⁰¹ has recently pointed out the heart should be carefully watched regarding its force and regularity.

Knowles considers that a 2 gramme course of this drug distributed over a period of 3 months is sufficient to sterilize an adult of parasites.

Deaths have occurred as the direct result of the use of tartar emetic.

Knowles in India has recorded five deaths in twenty treated cases, and fatal results have also occurred in the Sudan.

The writer has recently recorded the pathological findings in a fatal case of bilharzia treated by tartar emetic injections. Marked fatty changes in the organs and blood vessels were noted in this case (Plates 13 & 14), and it is reasonable to suppose that fat embolism was the cause of sudden death in this particular instance.

Tartar emetic may also be given intramuscularly, and by the mouth. Muir supplements the intravenous injections by giving antimony by the mouth. He gives tartar emetic orally in combination with tannic acid and sodium bicarbonate. For children and nervous patients Rogers recommends an ointment of metallic antimony in lanoline (5-10 per cent).

NAME *Malmed Shra*

ADDRESS

OCCUPATION

AGE

CASE BOOK No

PAGE

DISEASE

MALA AZAR

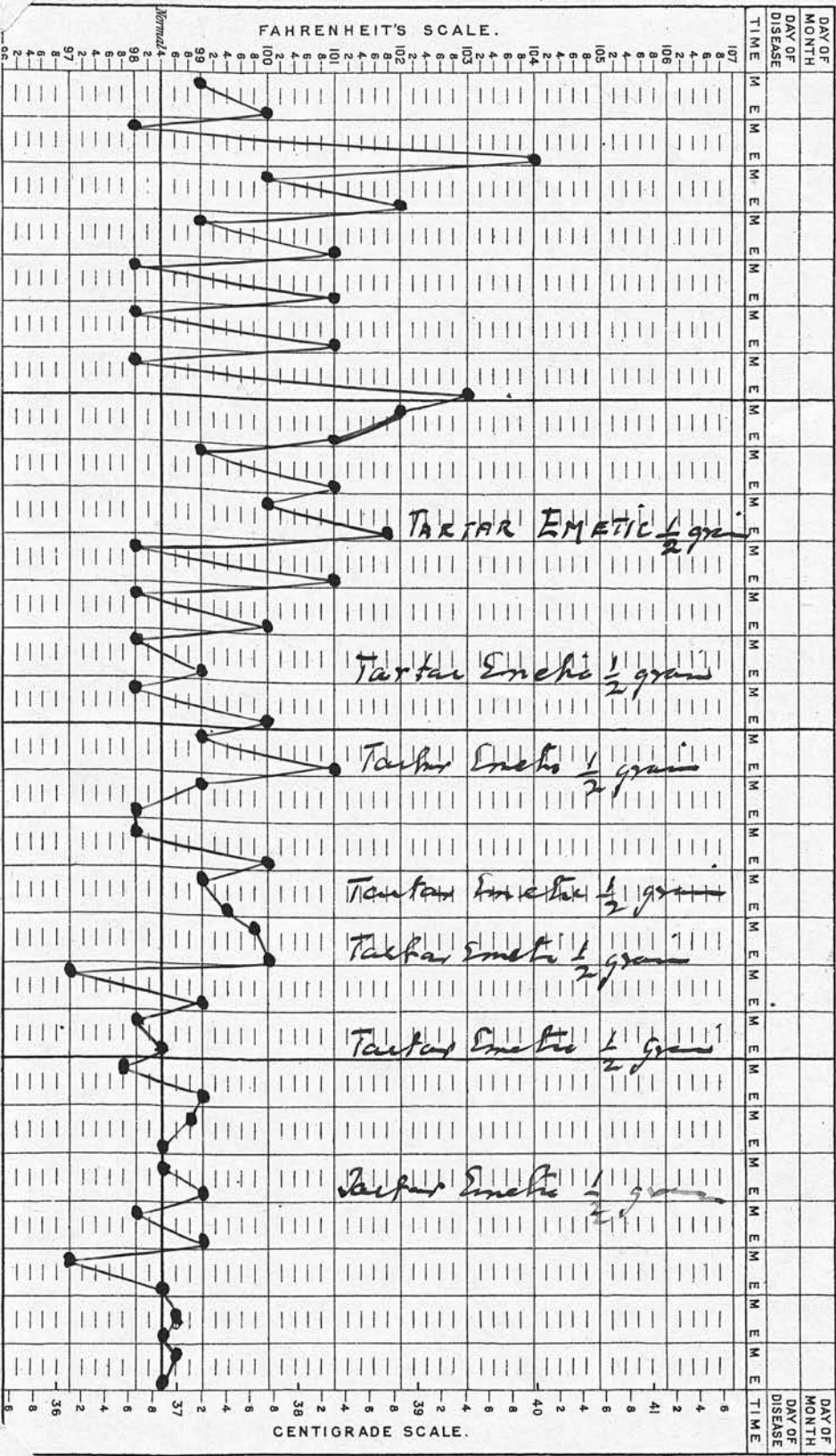


Chart illustrating the value of tartar emetic (with nausea induced) in the treatment of heart failure. Prior to treatment patients had a high venous pressure for several weeks.

102

Brahmachari recommends colloidal metallic antimony given in doses of .001 grammes for intramuscular injections and .002 grammes for intravenous injections. Colloidal metallic antimony appears to be less toxic.

The therapeutic ~~value~~ of antimony in the treatment of kala azar is shown by its beneficial action on the temperature. The ~~drug~~ also causes a diminution in the size of the spleen and liver ; films preparations of smears obtained by puncture of these organs will demonstrate the destructive action of the drug on the causal parasites. Whatever form of antimony treatment is employed, it is necessary to improve the general condition of the blood by the exhibition of iron tonics. To combat the leucopenia the internal administration of yeast is advisable, and for the existing asthenia, a nourishing diet and massage will prove helpful. The development of complications seriously influences the prognosis, and should be dealt with assiduously and energetically.

Among other drugs that have been used in the treatment of kala azar mention may be made of arsenic. It has been employed in various forms, and in some cases apparently, has temporarily arrested the progress of the disease but there is no record of recovery attributable to its action.

Dioxydiamidoarsenobenzol or "606" was proved by Nicolle
103

and Conor to be successful in killing the parasites in

104

artificially infected dogs. The writer gave this preparation a trial in a case of Sudan kala azar ; the result obtained was encouraging though not conclusive.

The tartarite of Bismuth given intravenously in 1 per cent solutions in distilled water has been recently employed by

by Brahmachari and the results have been promising.

Other drugs have been used in the treatment of this disease many of them with indifferent success, and a summary of them is given in a useful brochure by Brahmachari.

Vaccines prepared from cultures of *L. donovani* have been given a trial but the results have been disappointing in the hands of Cristina ¹⁰⁵, Lonigo ¹⁰⁶, Cornwall ¹⁰⁷ and others.

Cornwall has recently shown that extracts of *L. donovani* flagellates do not stimulate the formation of antibodies. The writer ¹⁰⁸ treated a case in the Sudan with apparently favourable results, by injections of an autogenous sensitized vaccine prepared from a six day's culture of flagellates, and Bassett Smith ¹⁰⁹ has also recorded a case in which vaccines were employed with beneficial results.

The operation of splenectomy has been recommended and tried in the treatment of kala azar.

Melissinos has recorded a case of complete recovery following splenectomy, but other cases in which this operation has been performed have not been attended with such happy results.

It is somewhat difficult to follow the rationale of splenectomy in such a systemic disease as kala azar, moreover the severity of such an operation, and the doubtful results following it are certainly points which scarcely recommend it as a line of treatment.

Conclusions.

The main facts embodied in this paper may be briefly summarized :

1. Visceral Leishmaniasis or Kala Azar has been recorded in Asia, Africa and certain parts of Europe. In Asia it has been found in India, Ceylon, China, Turkestan and Asia Minor ; in Africa the disease exists in Tunis, Algeria, Abyssinia and the Soudan ; in

Europe it has been recorded in Italy, Greece, Malta, Spain and Portugal.

2. In the Sudan the disease has a limited endemicity being confined to the Kassala, Sennar and Blue Nile Districts, and occurs in villages dependent on rivers or khors for a water supply.
3. The disease in the Sudan does not occur in epidemics; is more common in late childhood and young adults, and rarely occurs in females. No records exist of two members of the same family or occupying the same house acquiring the disease.
4. The disease has a seasonal incidence most of the cases acquiring infection shortly after the rainy season and developing symptoms after an ^{incubation} ~~uncertain~~ period approximating 60 days.
5. Clinically and pathologically the Sudan type closely resembles the Indian type, and no cytological or cultural differences in the causal parasites have been noted.
6. The parasites are rarely to be found in the peripheral blood of Sudan cases.
7. Two types of the disease exist in the Sudan.
8. Cultural and animal experiments failed to show any differences in the causal parasites obtained from the two types.
9. Attempts to prove the transmitting agent of the disease have failed, as in other countries.
10. Epidemiological and experimental investigations do not support the theory that the disease in the Sudan is insect borne.
11. No natural hosts of Leishmania parasites have been found either in the animals or insects of the Sudan.
12. The disease has been transmitted to animals by feeding experiments.

13. Animal experiments have shown that the Sudan virus can be transmitted to the grey monkey, pup (with difficulty) jerboa and gerbil.
14. Dogs are infected with difficulty, have never been found naturally infected and in the Sudan can scarcely be considered as carriers of the disease.
15. Animal experiments have shown the similarity existing between the Indian and the Mediterranean virus, and immunity experiments have furnished further proof in support of the view, that Indian and Mediterranean kala azar is caused by the same virus.
- 16@ Immunity experiments are necessary to prove that the Sudan virus is identical with the Indian and Mediterranean virus.
17. Experiments carried out with cultures of ^{Sudan}~~Indian~~ strains show that cultural forms of the parasite are endowed with fair powers of resistance, but are unable to survive in the presence of human serum, which suggests that the parasite is not transmitted to man in any cultural form.
18. Anomalous types of the disease exist in the Sudan.
19. The therapeutic value of Antimony has been proved in Sudan kala azar.

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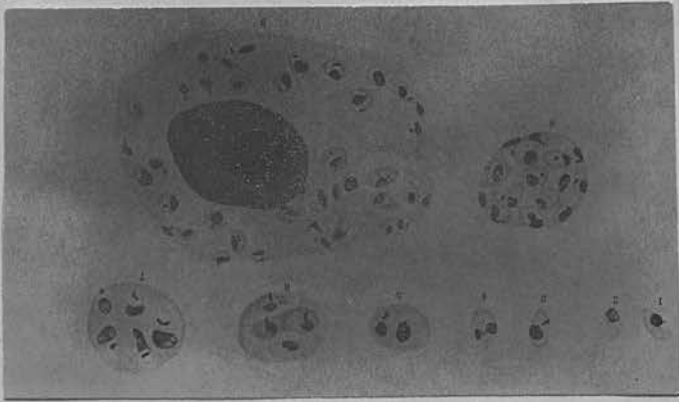
P h o t o g r a p h s .

- Plate 1. Photomicrograph of spleen smear showing free and phagocyted Leishmania parasites from a case of Sudan Kala Azar.
- Plate 2. Photomicrograph of a six days' culture showing fully developed flagellates and flagellates reverting to oval or cystic forms.
- Plate 3. Case of Sudan Kala Azar (acute type) showing lower border of spleen (marked in white).
- Plate 3a. The same case showing enlargement of the liver.
- Plate 4. Case of Sudan Kala Azar affecting an adult.
- Plate 5. Case of Sudan Kala Azar showing abdominal distension.
- Plate 6. Portion of large intestine showing ulceration from a fatal case of Sudan Kala Azar.
- Plates 7 & 7a. Grey monkey showing local lesion over the left eyebrow the result of vaccination with infected splenic material.
- Plate 8. Grey monkey showing local lesion on the inner surface of right thigh following a subcutaneous injection of infected spleen liver and bone marrow.
- Plate 9. Grey monkey showing enlargement of the spleen (mapped out in black) in experimental Leishmaniasis.

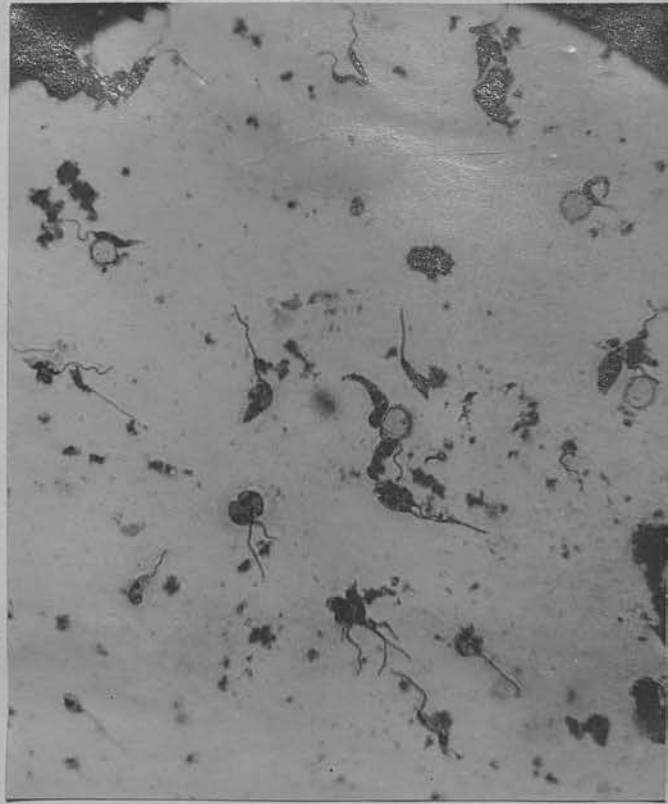
P h o t o g r a p h s (continued).

- Plate 10. Herpetomonas flagellate (H. lygaei)
 showing various stages of reversion to
 small oval or cystic forms.
- Plate 11. Liver smear showing intracellular coccal
 bodies from a case representing an
 anomalous type of Kala Azar.
- Plate 12. Grey monkey infected experimentally by
 feeding. Margins of the enlarged
 spleen depicted in black.
- Plate 13. Photomicrograph of a liver section stained
 by Marchi's method to show fatty changes
 in the liver cells, the result of intra-
 venous injections of Tartar emetic in
 a case of Bilharzia.
- Plate 14. Photomicrograph of section of kidney from
 the same case showing fatty changes in
 the tubules.

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3a.



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7a.



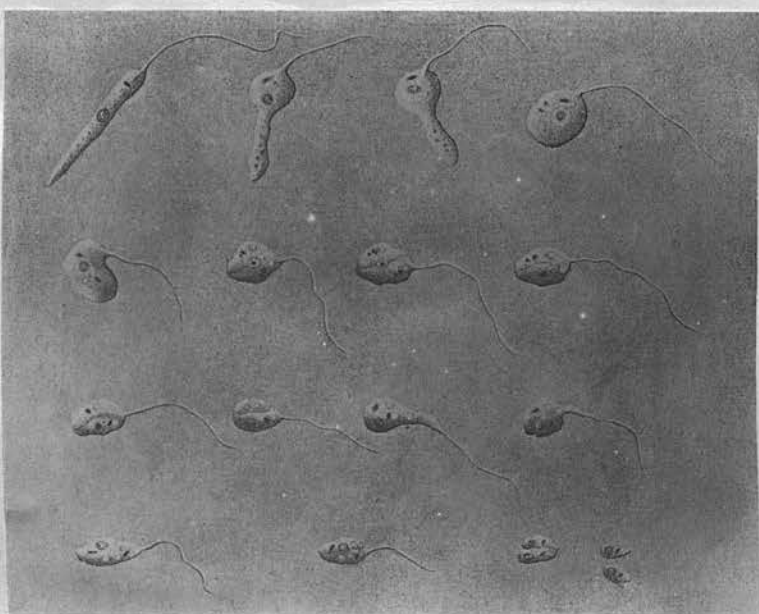
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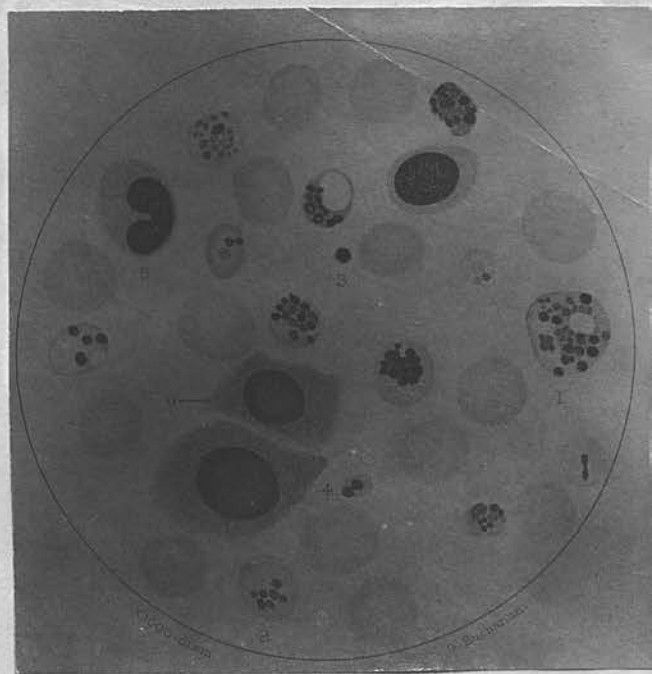
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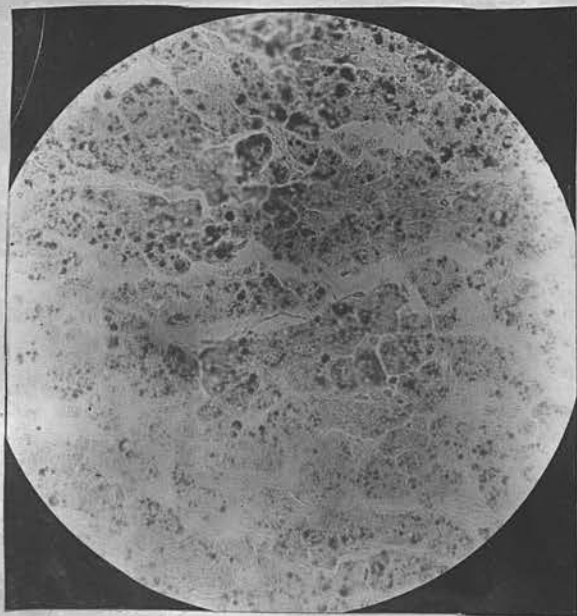
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